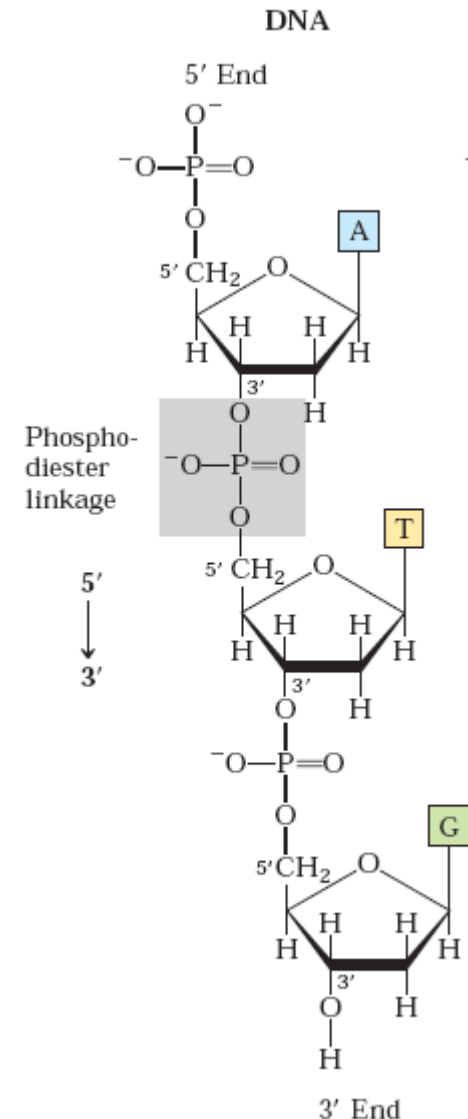


DNA ISOLATION AND GEL ELECTROPHORESIS

Lucia Dhiantika Witasari

Physical Characteristics of DNA

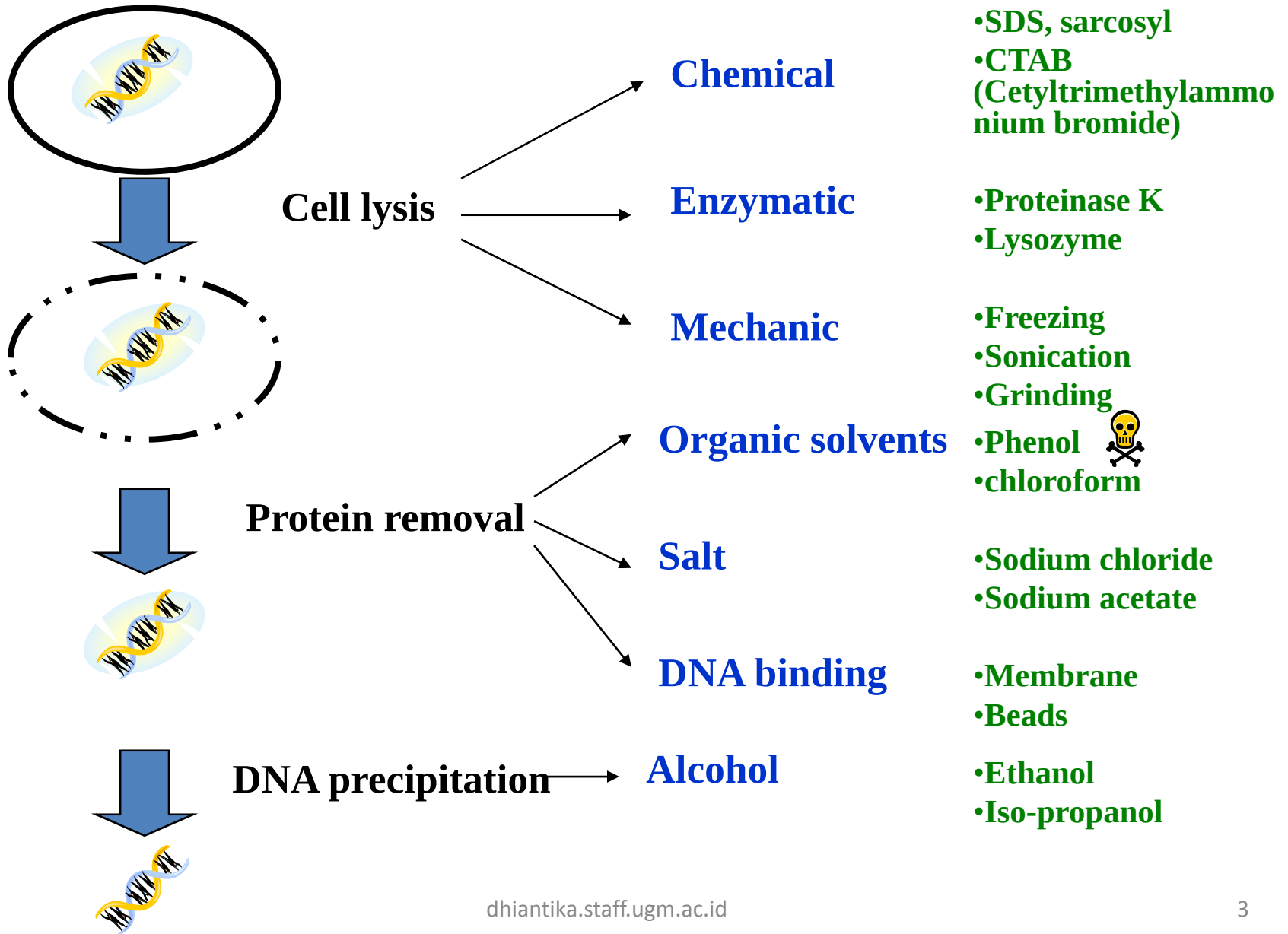
- DNA absorbs UV light at 260 nm
 - Allows quantitation
- DNA is water soluble
- DNA precipitates in alcohols
- DNA carries a net negative charge
- DNA has characteristic melting & annealing temperatures



DNA extraction – the basic concept

Process

Common procedure



PLASMID DNA ISOLATION

1 A culture of bacteria is grown and then harvested



Centrifugation

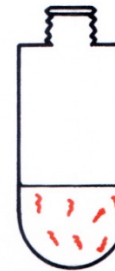


Lysis I, Lysis II
Lysis III



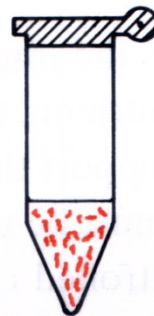
Cell extract

Phenol-chloroform-isoamylalcohol extraction



Pure DNA

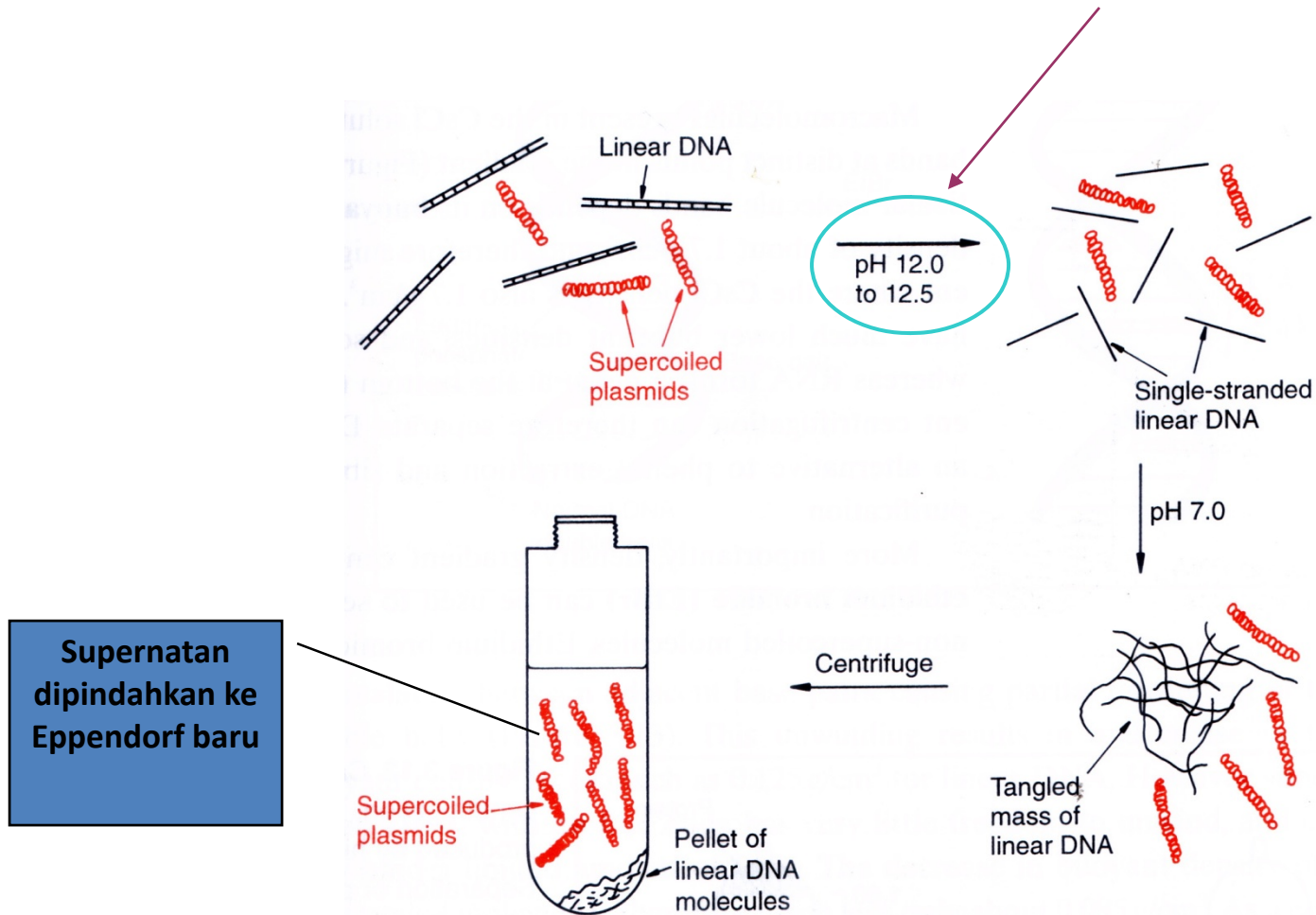
Et-OH

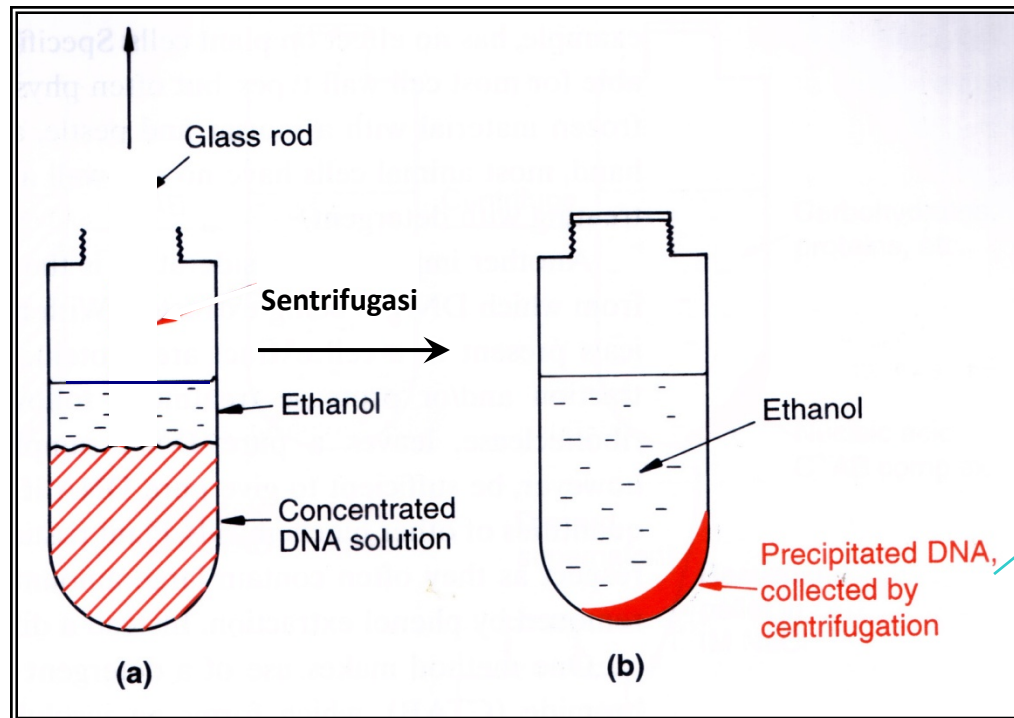
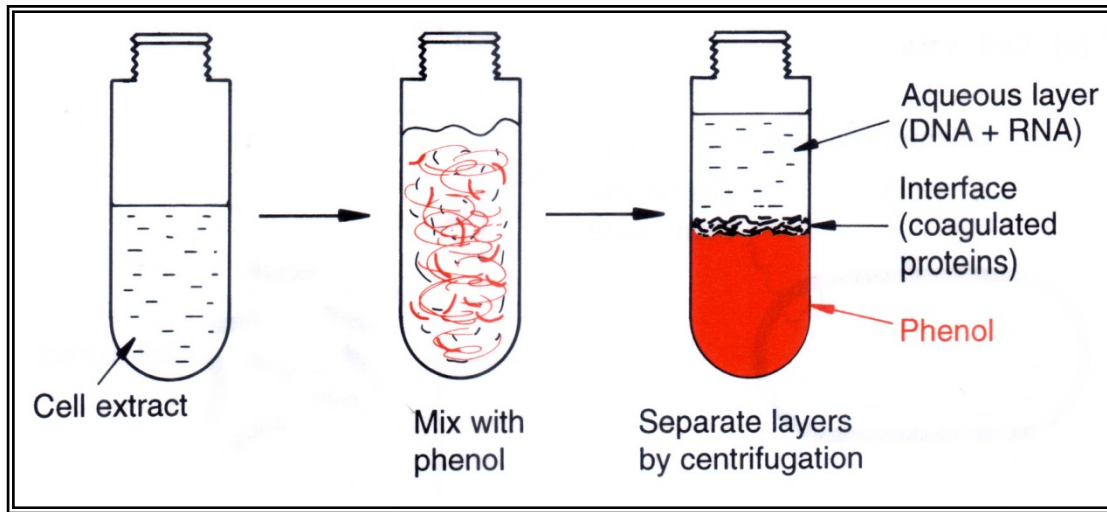


4 The DNA is concentrated

3 The DNA is purified from the cell extract

Gambaran saat penambahan SDS NaOH





**DNA & RNA
Dilarutkan TE**

Extraction/Lysis Buffers and Solutions

Alkaline Lysis Solution I (Plasmid Preparation)

- 50 mM glucose
- 25 mM Tris-Cl (pH 8.0)
- 10 mM EDTA (pH 8.0)

Prepare Solution I from standard stocks in batches of ~100 ml, autoclave for 15 minutes at 15 psi (1.05 kg/cm²) on liquid cycle, and store at 4°C.

Alkaline Lysis Solution II (Plasmid Preparation)

- 0.2 N NaOH (freshly diluted from a 10 N stock) <!=>
- 1% (w/v) SDS

Prepare Solution II fresh and use at room temperature.

Alkaline Lysis Solution III (Plasmid Preparation)

- | | |
|--------------------------|---------|
| 5 M potassium acetate | 60.0 ml |
| glacial acetic acid <!=> | 11.5 ml |
| H ₂ O | 28.5 ml |

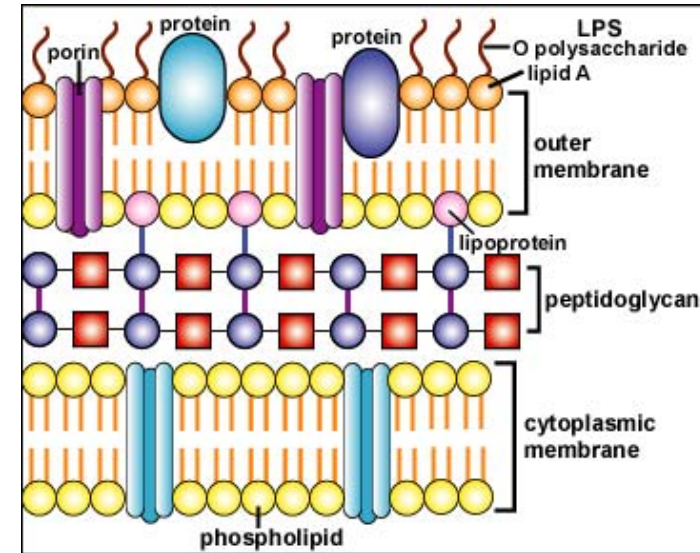
The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the solution at 4°C and transfer it to an ice bucket just before use.

CHROMOSOMAL DNA ISOLATION

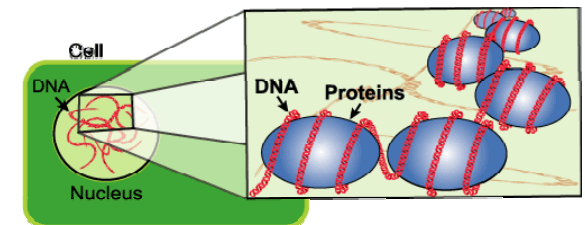
Extract and purify DNA

Resuspend & lyse bacterial cell walls.

- Resuspend cells in Ice cold TES (Tris, EDTA, NaCl)
- lysozyme (degrades peptidoglycan cell walls)
- Proteinase K (to degrade protein that are bound to the DNA molecule)
- Enzymes (RNAses) are often added to degrade the RNA in the sample for purification
- SDS (detergent to lyse the cells)



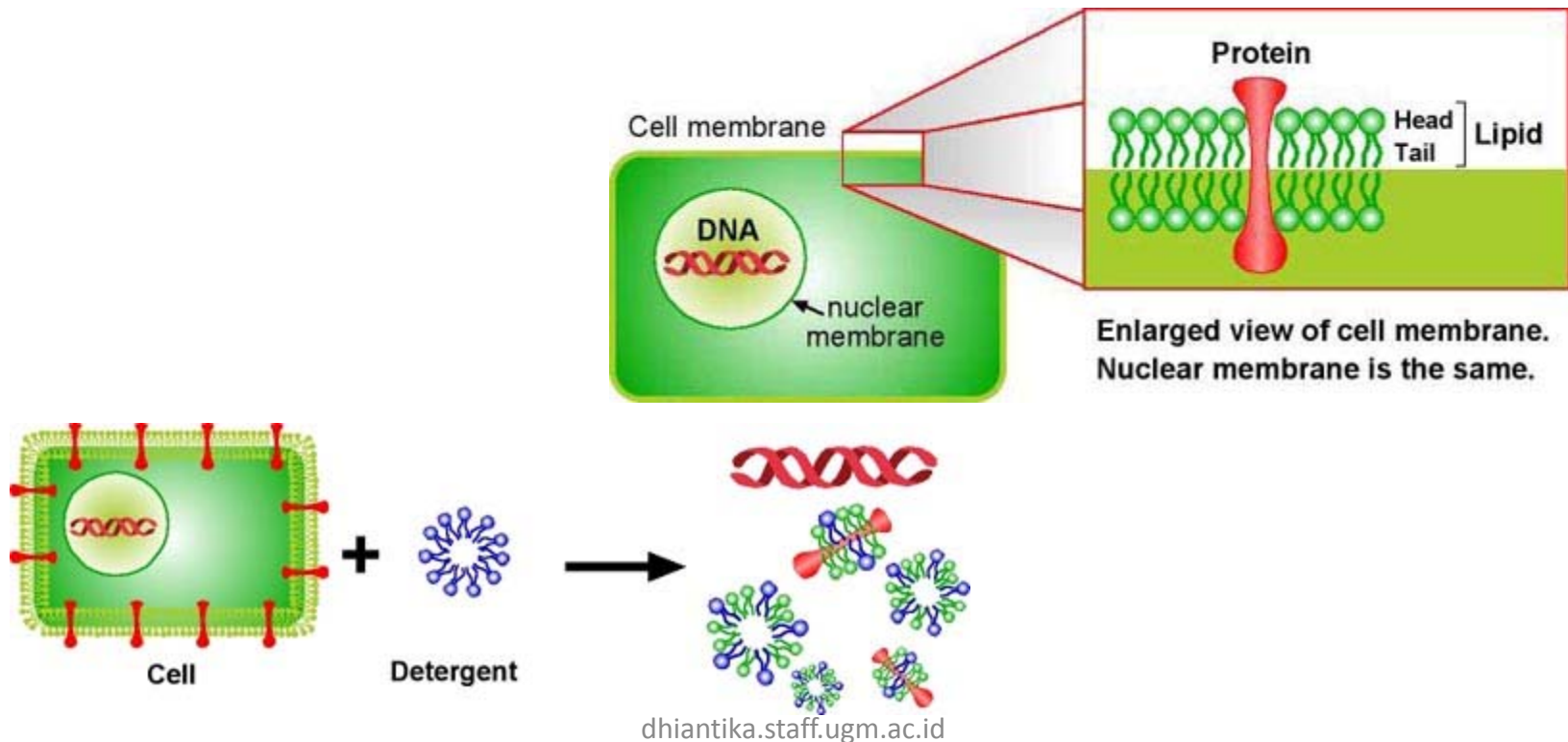
Structure of a Gram-Negative Cell Wall



The DNA in the nucleus is surrounded by proteins

Detergent:

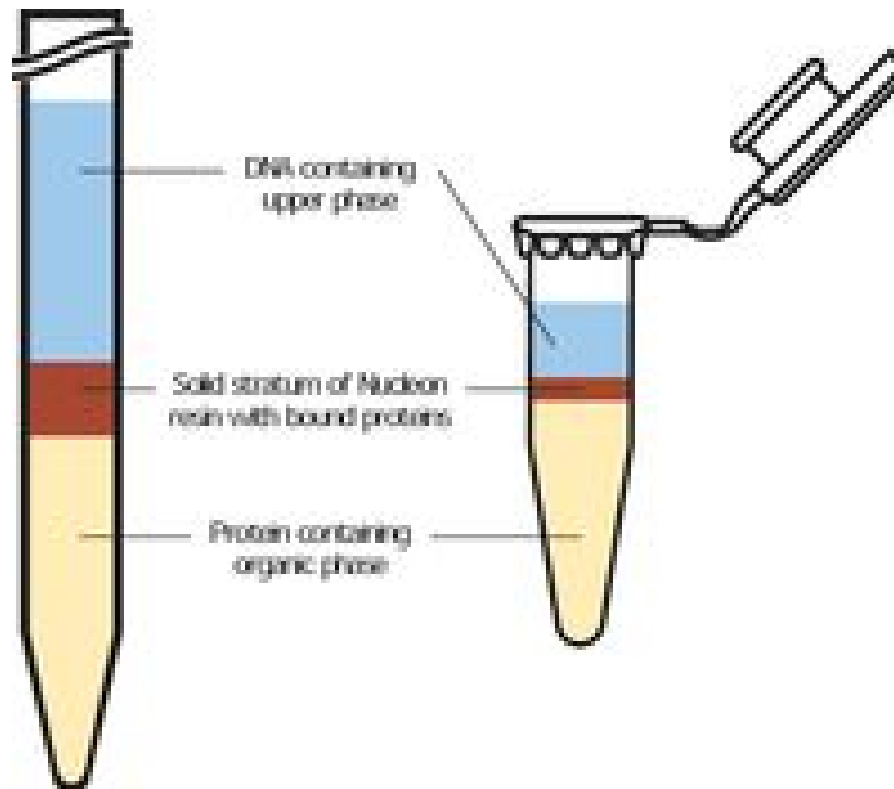
- Breaks apart membranes by attaching to the lipids (fats) & proteins in the membranes



Extract and purify DNA

c. Separation of DNA from other molecules

– Phenol extraction



The cell and nuclear membranes have been broken apart,
as well as all of the organelle membranes,
such as those around the mitochondria and
chloroplasts.

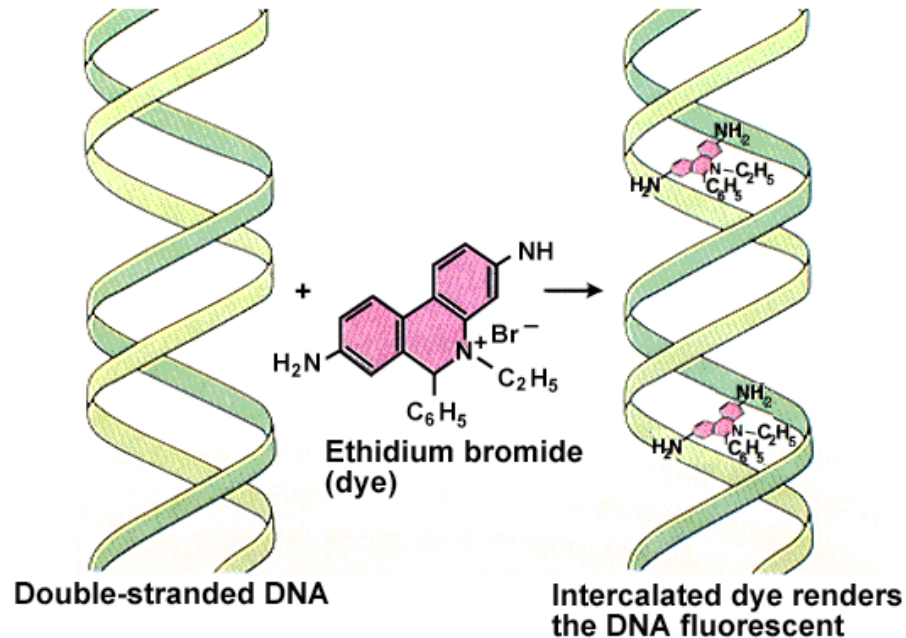
So what is left?

- Proteins
- Carbohydrates (sugars)
- DNA

DNA isolation

- DNA and RNA have similar physical properties
- Enzymes (RNAses) are often added to degrade the RNA in the sample for purification
- Enzymes (proteases) are also added to degrade protein that are bound to the DNA molecule

Fluorescent Dye for DNA

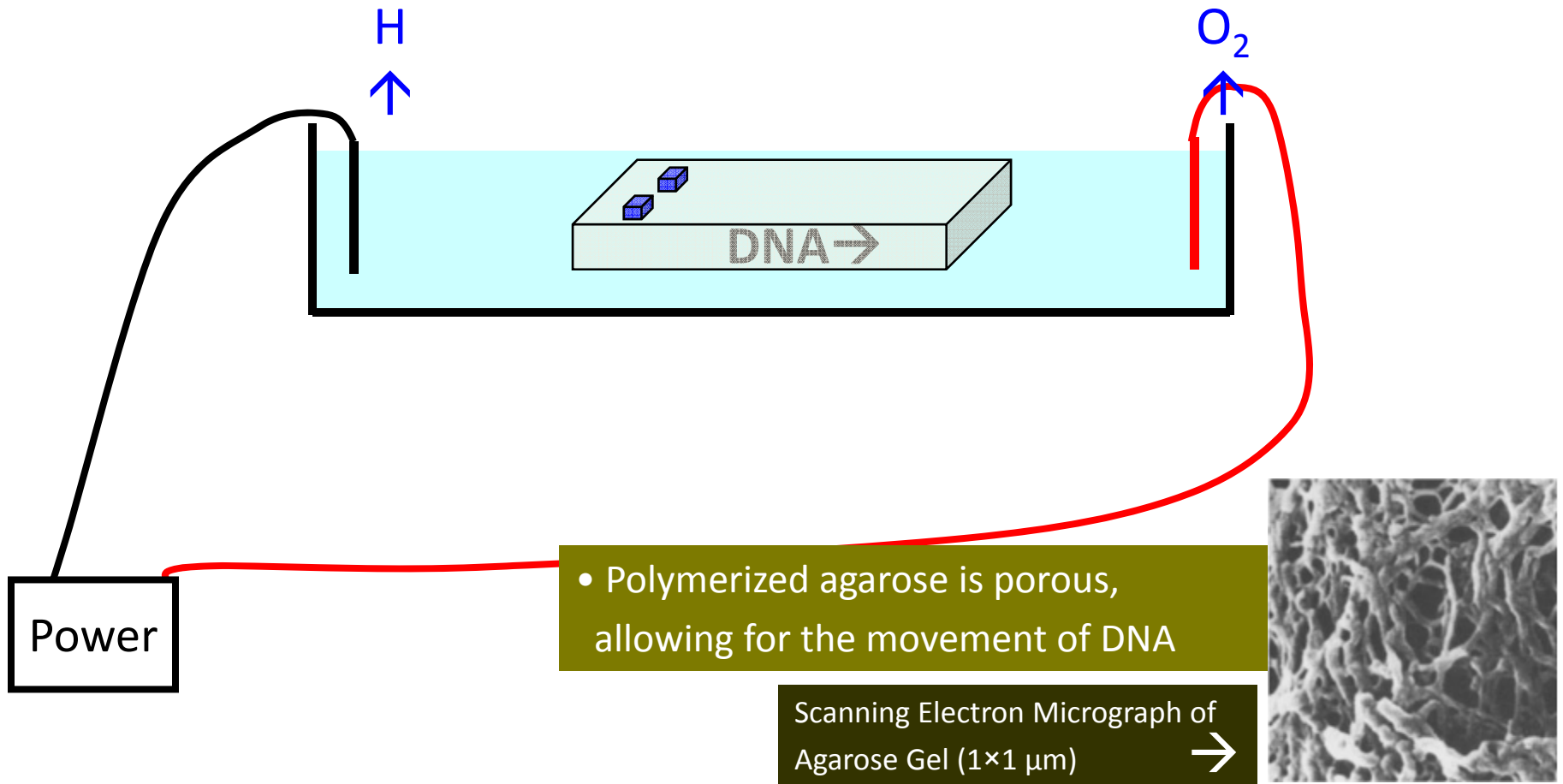


- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.
- Ethidium bromide can be added to the **gel** and/or **running buffer** before the gel is run or the gel can be stained after it has run.

GEL ELECTROPHORESIS

- Gel electrophoresis is a widely used technique for the analysis of **nucleic acids** and **proteins**. Agarose gel electrophoresis is routinely used for the preparation and analysis of DNA.
- Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field.

- DNA is negatively charged.
- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel is used to slow the movement of DNA and separate by size.



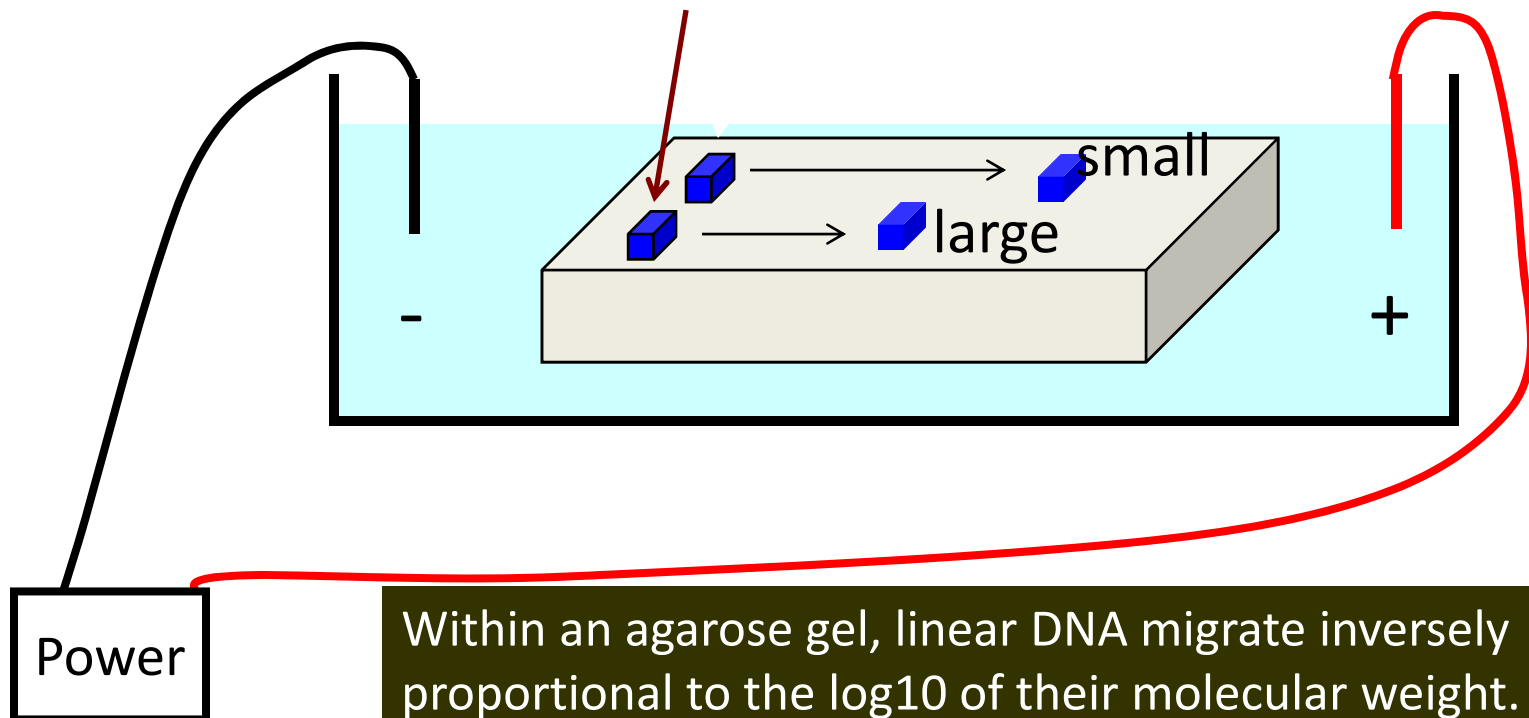
How fast will the DNA migrate?

strength of the electrical field, buffer, density of agarose gel...

Size of the DNA!

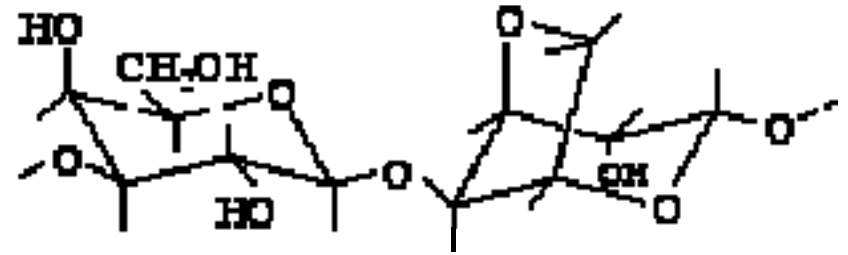
*Small DNA move faster than large DNA

...gel electrophoresis separates DNA according to size



Within an agarose gel, linear DNA migrate inversely proportional to the $\log_{10}$ of their molecular weight.

Gel types



- Agarose is isolated from seaweed and highly purified
- Mainly used for DNA size analysis and for DNA separation prior to blotting
- Percentage of agarose in gel affect size separation
- Increase % increased separation of small fragments

Commonly Used Electrophoresis Buffers

Buffer	Working Solution	Stock Solution/Liter
TAE	1x 40 mM Tris-acetate 1 mM EDTA	50x 242 g of Tris base 57.1 ml of glacial acetic acid <!--> 100 ml of 0.5 M EDTA (pH 8.0)
TBE ^a	0.5x 45 mM Tris-borate 1 mM EDTA	5x 54 g of Tris base 27.5 g of boric acid 20 ml of 0.5 M EDTA (pH 8.0)

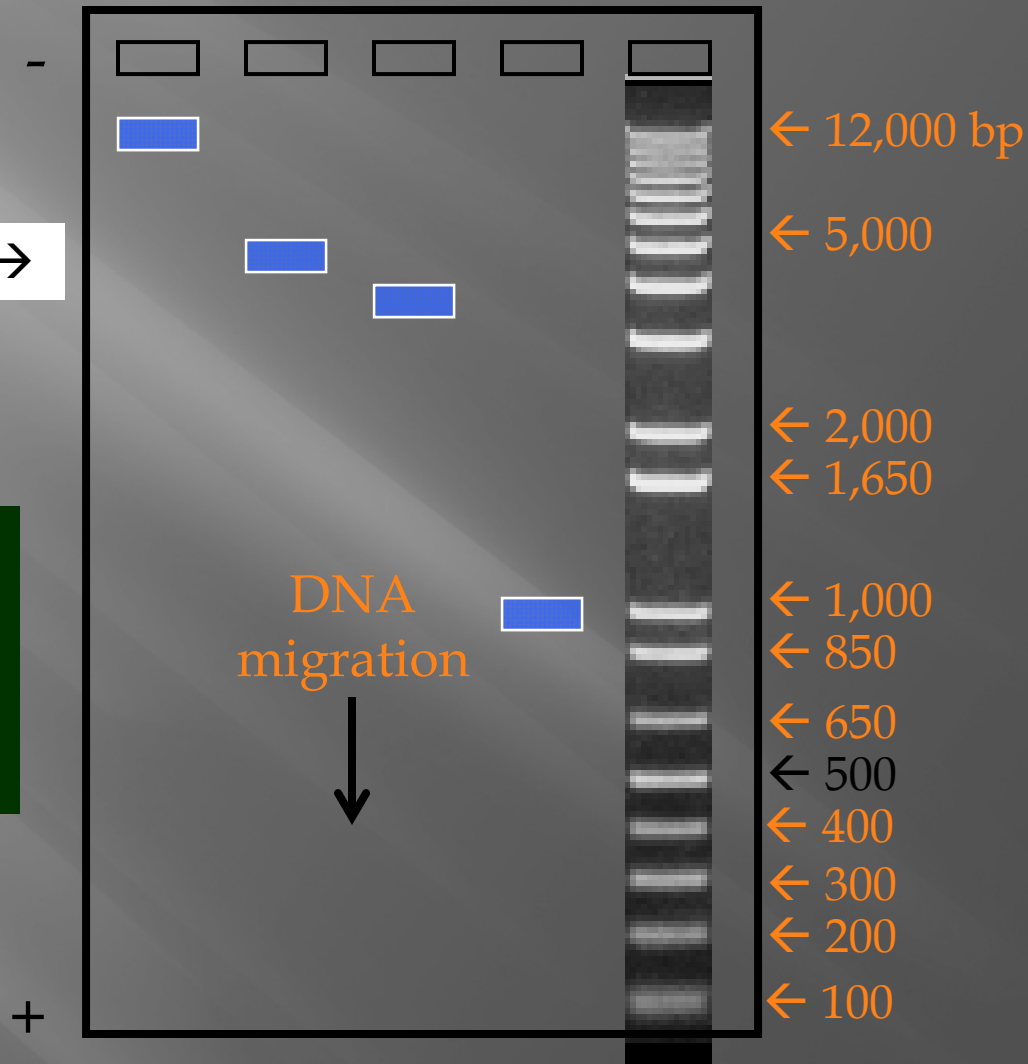
Detection of DNA

- DNA can be detected by staining or labeling
- Commonly stained by adding ethidium bromide or silver staining
- Can be labeled with radioactive or fluorescent tagged nucleotides
- Often involves a blotting procedure

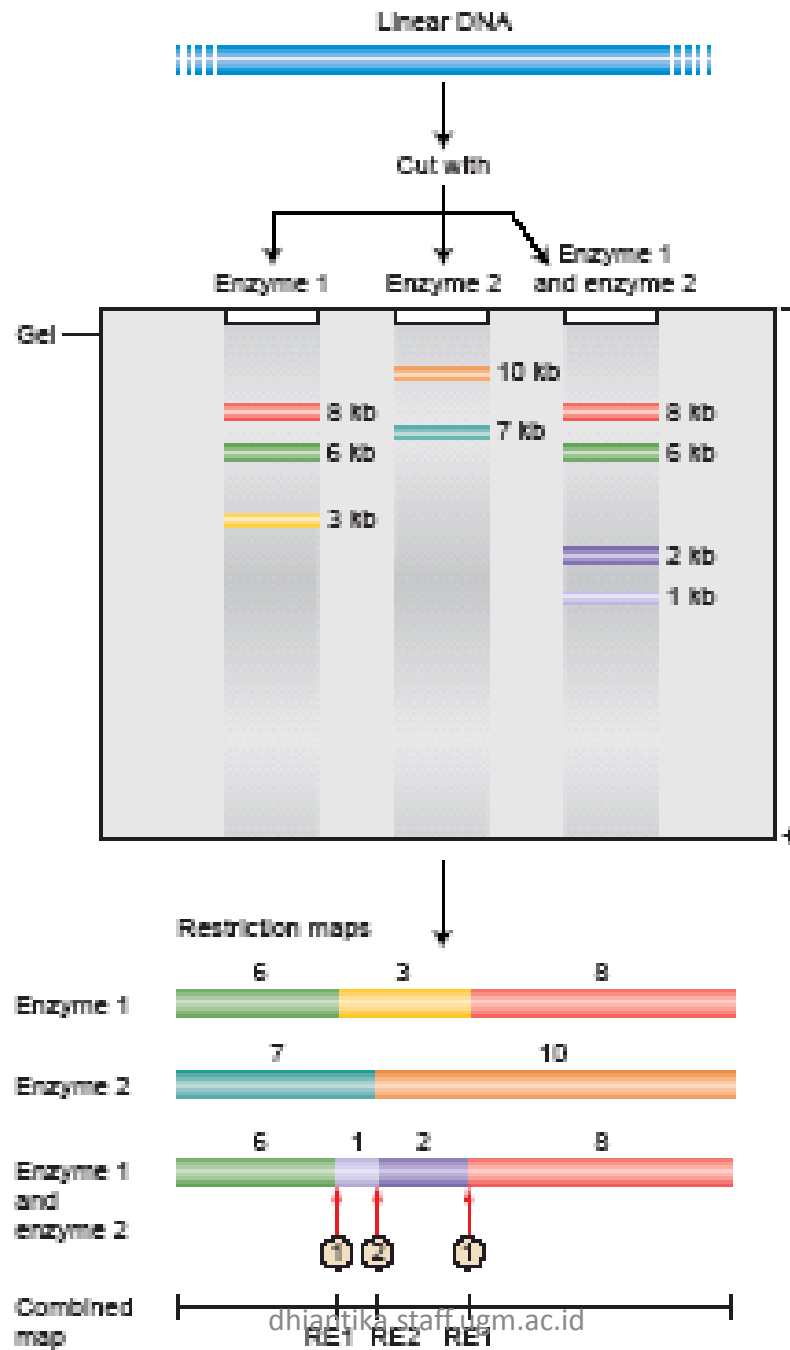
DNA Ladder Standard

bromophenol blue→

Note: bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule



Inclusion of a DNA ladder (DNAs of known sizes) on the gel makes it easy to determine the sizes of unknown DNAs.



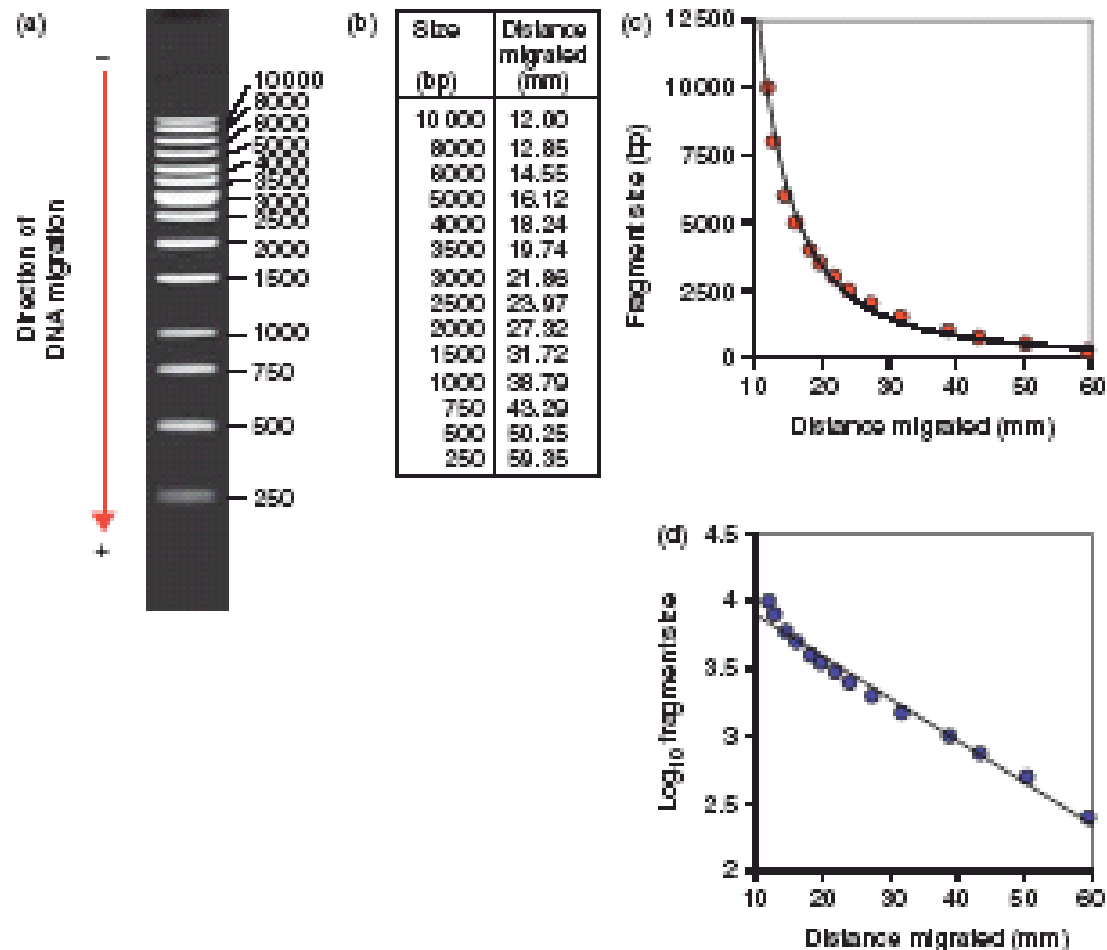


Figure 2.13. The migration of DNA fragments through an agarose gel. (a) An agarose gel showing the separation of DNA fragments of known size. (b) The size of a DNA fragment and the distance migrated through the gel. (c) A plot of fragment size against distance migrated taken from the data shown in (b). This indicates that the relationship between fragment size and distance migrated is not linear. (d) A plot of the log of fragment size against distance migrated. This indicates that there is a direct, inverse, relationship between distance migrated and the log of the size of a DNA fragment

Analyzing DNA

Kualitas DNA

Konsentrasi tinggi

Utuh, tidak terputus-putus

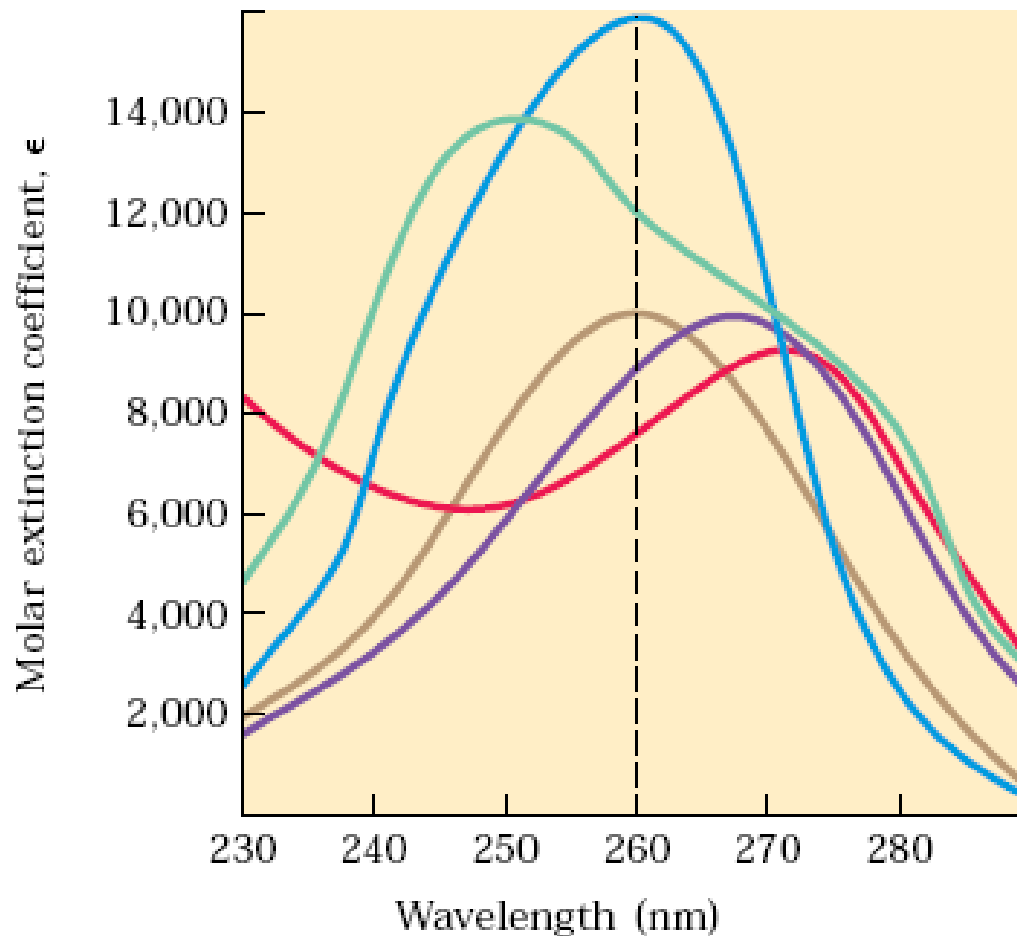
Tidak banyak terkontaminasi oleh protein

Menentukan Kualitas DNA

Dengan Spektrofotometer

Dilihat dengan elektroforesis, band yang tebal secara kualitatif menunjukkan DNA yang bagus

Wavelength of DNA



Molar extinction coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)		
AMP	15,400	
GMP	11,700	
UMP	9,900	
dTMP	9,200	
CMP	7,500	

Using Spectrophotometer

Concentration of DNA

- 1 A_{260} Unit of dsDNA = 50 $\mu\text{g/ml}$
- 1 A_{260} Unit of ssDNA = 33 $\mu\text{g/ml}$
- OD value should range between 0.1 and 1.0 to ensure an optimal measurement.

Example of calculation:

- Volume of dsDNA sample: 100 μl
- Dilution: 25 μl of sample + 475 μl H₂O (1+19 dilution)
- A_{260} : 0.44
- Concentration of dsDNA: $0.44 \times 50 \mu\text{g/ml} \times 20 = 440 \mu\text{g/ml}$
- Amount of dsDNA: $440 \mu\text{g/ml} \times 0.1 \text{ ml (sample volume)} = 44 \mu\text{g}$

Purity of DNA

- Pure DNA: $A_{260}/A_{280} > 1.8$
- An $A_{260}/A_{280} < 1.8$ indicates that the preparation is contaminated with protein and aromatic substances.
- An $A_{260}/A_{280} > 2$ indicates a possible contamination with RNA.
- The OD gives no information about the size of the DNA